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Comparison of two different culture systems for human articular chondrocytes.

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Purpose: The cell culture method is an essential step in the Autologous Chondrocyte Implantation (ACI) treatment; however studies involving different culture systems have been limited. This study compares two different cell culture systems. An explant system (ES), and the collagenase (enzymatically) method (CM).
Methods and Materials: Materials and Method: Four (4) cartilage biopsies were used. The viability of cells generated after CM was determined by trypan blue exclusion. Morphological differences were evaluated by light microscopy analysis. Cells were analysed with flow cytometry and RT-PCR. Antibodies against CD44, CD73 and Stro-1 were used for flow cytometry. Specific primers for collagen type II, collagen type I, aggrecan and sox9 were used for the RT-PCR.
Results: The cell fraction obtained by CM was highly apoptotic. The CM resulted in colonies, which were highly confluent in the centre. Likewise the EM resulted in colonies, however without a confluent centre. A clear difference in cell morphology was observed. In the gene expression analysis no differences were observed. With the EM a higher fraction of CD44 positive cells were obtained. The CD73 positive fraction was higher with the CM. No difference was observed with Stro-1.
Conclusions: Initially, the CM resulted in a highly apoptotic cell fraction. The highly confluent colonies obtained with CM, could limit the nutrient supply. A high fraction of CD44 positive cells was obtained with EM. These results indicate that the EM leads to more viable chondrocytes with higher potential for surviving implantation. This suggests that the EM could be advantageous for culturing chondrocytes for ACI.

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SIRT1, longevity factor and class III histone deacetylase, regulates the apoptosis of human chondrocytes.

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Purpose: SIRT1, a mammalian homologue of longevity factor sir2, is known to inhibit apoptosis and promotes cell survival in cardiac myocytes and neuron. Yet there have never been reported the expression of SIRT1 in human primary chondrocytes (HCs). The purpose of this study is to investigate the localization of SIRT1 in HCs and to elucidate the relations of SIRT1 with apoptosis of HCs.
Methods and Materials: Expression of SIRT1 in HCs was examined by RT-PCR and immunoblotting. HCs were transfected with SIRT1-GFP plasmid constructs by electroporation method and cellular localization of SIRT1 were examined in confocal microscopy. The expressions of SIRT1 under stresses, such as oxidative, mechanical, and nutritional stresses in HCs were examined by immunoblotting. Effects of SIRT1 inhibitors, sirtinol and nicotinamide in HCs were examined by immunoblotting. TUNEL staining was performed to examine whether SIRT1 is related with apoptosis in HCs.
Results: Expression of SIRT1 was detected by RT-PCR and western blotting in HCs. Human GFP tagged SIRT1 was detected in the nuclei in HCs. However, GFP, as a control vector, was detected in the perinuclear plasma. All oxidative, mechanical, and nutritional stresses inhibited SIRT1 expressions in HCs. SIRT1 inhibitors suppressed SIRT1 expression in dose-dependent manner. SIRT1 inhibitors significantly induced apoptosis in HCs in TUNEL staining.
Conclusions: SIRT1 was expressed in the nucleus of HCs. The results of this study indicated that SIRT1 regulates the apoptosis in HCs. Further research of SIRT1 might contribute to resolve the pathogenesis of osteoarthritis.

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Neither Notch1 expression nor cellular size correlate to mesenchymal stem cell properties of adult articular chondrocytes

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Purpose: Tissue regeneration and repair are thought to be regulated by progenitor cells, which in other tissues are characterized by their Notch1 expression or small cellular size. Here we have studied if these traits affect the chondrogenic potential or are markers for a multipotent progenitor cell population in adult articular cartilage.
Methods and Materials: Directly isolated articular chondrocytes were sorted with regard to their Notch1 expression or cellular size using flow cytometry. Their colony forming efficiency (CFE) was investigated as well as their potential to differentiate towards adipogenic, osteogenic, and chondrogenic lineages. The different sorted populations were also expanded in monolayer and analyzed in the same manner as the directly isolated cells.
Results: Our results showed that there are no differences in CFE or adipogenic, osteogenic, and chondrogenic potential between the sorted populations, indicating that neither Notch1 nor cellular size are markers for a mesenchymal progenitor cell population in adult articular cartilage. Expanded cells displayed a higher osteo-chondral potential than directly isolated cells. These results demonstrate that monolayer expanded adult chondrocytes contain a larger mesenchymal progenitor cell-like population than directly isolated cells, highly likely as a result of de-differentiation
Conclusions: In conclusion, cellular size or Notch1 per se are no markers for mesenchymal progenitor cells in adult articular cartilage nor have higher chondrogenic potential. If there are resident cells in adult articular cartilage with functional features of progenitor cells, the population consists of only a very small number of cells.

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Shear stress induced apoptosis of human chondrocytes via p53 pathway

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Purpose: Chondrocyte apoptosis plays an important role in cartilage degeneration of Osteoarthritis (OA), and the mechanical injury to explants of cartilage introduce chondrocyte apoptosis. In response to DNA damage, p53 expression is upregulated and was increased transcriptional activity. Then, the cell-cycle arrested or started apoptosis signal. In this study, we investigated the analysis of p53 function in apoptosis of chondrocytes introduced by shear stress.
Methods and Materials: Cartilage tissues were obtained during joint surgery. Chondrocytes were isolated from human cartilage tissues and cultured. The expression and localization of p53 in OA cartilage were analyzed by immunohistochemistry. The expression of p53 in OA and normal chondrocytes were analyzed by RT-PCR and western blotting. Shear stress was introduced to NHAC-kn cells (human normal chondrocyte) with Flexercell system. After induction of shear stress with or without incubation of pifithrin-alfa (p53 specific inhibitor), chondrocytes apoptosis were detected by western blotting and TUNEL staining, and expression levels of p53 were analyzed by RT-PCR and western blotting.
Results: Immunohistochemistry demonstrated that p53 is expressed in the chondrocytes mainly in superficial zone of OA cartilage. p53 was expressed in OA and normal chondrocytes. TUNEL positive cells and the expression levels of p53 were increased by shear stress. TUNEL positive cells and the expression level of p53 were decreased when pifithrin-alfa were incubated.
Conclusions: We clarified that the expression of p53 was increased by shear stress, and played the important role in apoptosis of human chondrocyte. Pifithrin-alfa may prevent cartilage from apoptosis by inhibition of p53 activity.